Interactions Between Segmental Leg Central Pattern Generators During Fictive Rhythms in the Locust

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SUMMARY AND CONCLUSIONS

1. Rhythmic activity of leg motor neurons could be evoked in isolated locust thoracic ganglia as well as in preparations of two or three connected thoracic ganglia superfused with the muscarinic agonist pilocarpine. Rhythms were always more regular and reliably elicited in single isolated ganglia. When the ganglia were connected, rhythmic activity of leg motor neurons was not usually simultaneously evoked in all six hemiganglia. Typically, some of the hemiganglia were rhythmically active, whereas others showed tonic or highly irregular activity.

2. Action potentials from leg motor neuron pools were recorded extracellularly from motor nerves and cross-correlated with the use of standard algorithms. The following correlations were observed between activities of motor neurons in different hemisegments. 1) Within a segment, trochanteral levators were coactive with contralateral trochanteral depressors. This correlation was strong in the metathoracic ganglion, and weaker in the pro- and mesothoracic ganglia. 2) Coupling between levators on opposite sides of the same segment was variable in the pro- and mesothoracic ganglia, because phase relationships between levators were different in each preparation and could also change during the course of an experiment. In the metathoracic ganglion, levators on opposite sides were never coactive. 3) Trochanteral levators were often active within a short latency of levator bursts in an ipsilateral adjacent hemiganglion. In addition, levators in one segment were often inhibited during levator bursts in the ipsilateral adjacent segment. 4) Trochanteral levators were strongly coupled to ipsilateral adjacent trochanteral depressors, for all three thoracic ganglia.

3. The phase relationships between motor neuron activities revealed by cross-correlated are discussed in the context of what is known about the mechanisms involved in the control of intersegmental coupling during legged locomotion.

INTRODUCTION

The need to coordinate different body parts during locomotion is common to many species, invertebrates and vertebrates alike. Although it is generally accepted that neural circuits (central pattern generators) exist that can produce the basic motor patterns underlying movement of individual limbs or body segments in the absence of sensory inputs (Delcomyn 1980), little is known about how pattern generators in different body segments are coordinated during locomotion. The mechanisms underlying intersegmental coordination during locomotion are best understood in the context of locomotion in an aquatic environment (swimming) in both vertebrates (Grillner et al. 1991; Roberts et al. 1986) and invertebrates (crayfish: Paul and Mulloney 1986; Stein 1971; leech: Friesen 1989). In these systems the central pattern generator is composed of a chain of segmentally repeated pattern generators, each of which independently produces a rhythmic output appropriate for the movement of a single appendage or body segment. The coordination between body segments in nervous systems deprived of sensory feedback is similar to that observed in the intact animal, which indicates that intersegmental coordination can be achieved by central mechanisms alone (crayfish: Ikeda and Wiersma 1964; leech: Kristan and Calabresc 1976; Pearce and Friesen 1985; fish: Grillner et al. 1976; lamprey: Poon 1980; Wallén and Williams 1984; toad: Kahn and Roberts 1982). Although the neuronal basis of the intersegmental coordination is not completely understood in any of these systems, models have been proposed that can account for the coordination in terms of known circuit components such as intersegmental neurons and their connections (lamprey: Matsushima and Grillner 1992; leech: Friesen 1989).

Evidence for central control of intersegmental coordination in terrestrial walking is more scarce (reviews: arthropods: Cruse 1990; Graham 1985; vertebrates: Grillner 1981; Rossignol et al. 1994). Although segments of the nervous system can produce motor patterns appropriate for the movement of a single limb in the absence of sensory inputs (insects: Bässler and Wegner 1983; Pearson and Iles 1970; Ryckebusch and Laurent 1993; crayfish: Sillar and Skorupski 1986; cat: Grillner and Zangger 1979; chick: Jacobson and Hollyday 1982), coordination between individual limb pattern generators is often weak (Grillner and Zangger 1979) or behaviorally inappropriate (Bässler and Wegner 1983; Sillar and Skorupski 1986; Sillar et al. 1987). The most thorough studies of intersegmental coordination in an insect have been carried out in stick insects (review: Cruse 1990), but the relative influences of central and sensory mechanisms have not been investigated.

In locusts, we have recently developed a preparation in which sustained rhythmic activity appropriate for leg movement during walking can be evoked in motor neurons of isolated thoracic ganglia (Ryckebusch and Laurent 1993). This preparation now makes it possible to study the central mechanisms underlying intersegmental coordination of the legs in an isolated insect nervous system. Here we show evidence for central coordination of leg motor patterns in isolated locust thoracic nerve cords. Understanding the relative contributions of central and sensory mechanisms in...
generating appropriately coordinated leg movements should help us to gain insights not only about the nature of terrestrial walking, but also about how the nervous system integrates information from different sources to produce well-adapted behavior.

**METHODS**

**Electrophysiology**

Experiments were performed on 44 adult locusts *Schistocerca americana* of either sex, from our crowded laboratory colony. Cross-correlation analysis was performed on 30 different recorded segments of data (totaling 11,660 s) obtained from 12 of these preparations.

**PREPARATION.** Experiments were performed on an in vitro thoracic preparation described previously (Ryckebusch and Laurent 1993). Briefly, sections of the thoracic nerve cord consisting of two or three thoracic ganglia were removed from the thorax of the animal with the surrounding tracheal supply and air sacs undisturbed and were pinned down in a chamber lined with silicone elastomer (Sylgard 184; Dow Corning, Midland, MI). Leg motor nerves were carefully stripped of their surrounding connective tissue with a small hooked pin. The preparation was superfused with locust saline (in mM: 140 NaCl, 5 KCl, 5 CaCl₂, 4 NaHCO₃, 1 MgCl₂, 6.3 N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid, pH 7.0) supplemented with 2.5% (wt/vol) sucrose. Air was supplied to the ganglia by teasing open the tracheae at the surface of the saline. A stock solution of 10⁻² M pilocarpine hydrochloride (Sigma) was prepared in advance and was added to the saline to a final bath concentrations of 10⁻⁴–10⁻³ M. The preparation remained healthy for at least 4 or 5 h at 20–26°C.

**RECORDINGS.** The electrical activity of leg motor nerves was monitored extracellularly with the use of polyethylene suction electrodes. In all thoracic ganglia, recordings from trochanteral levator motor nerves were made from nerves 4A, and recordings from trochanteral depressor motor neurons were made from nerves 5A. Data were recorded on an eight-channel Digital Audio Tape recorder sampling at 5 kHz (Sony/Biologic) and displayed on a Gould TA4000 chart recorder.

**ANATOMY AND NOMENCLATURE.** The muscles are numbered according to Snodgrass (1929), except where a variant is now more commonly used in the literature. The nerves are numbered according to Bräunig (1982). Motor neurons are designated by commonly used abbreviations or by the name of the muscle group that they innervate. Detailed descriptions of the innervation of the leg musculature can be found in Campbell (1961), Bräunig (1982), and Siegler and Housman (1990).

**Data analysis**

For each extracellular recording all of the action potentials that could be resolved were used in the analysis. These recordings usually contained action potentials of several motor neurons from the same pool. Recordings of nerve 4A contained action potentials from up to 10 trochanteral levator motor neurons, and recordings of nerve 5A contained action potentials from 2 trochanteral depressor motor neurons (*D₄* and *D₅*) and a common inhibitory motor neuron (*CI*).

Electrophysiological recordings were analyzed off-line after digitization at 5 kHz with a National Instruments NBMIO16L AD/DA interface. The times of occurrence of action potentials were determined from the digitized extracellular recordings with the use of Spike Studio (E. Meir, University of Washington), and software written by W. Bair (California Institute of Technology) was used to compute cross-correlations between spike trains. Cross-correlations were computed with the use of standard algorithms (Glasner and Ruchkin 1976; Perkel et al. 1967) with a binwidth of 10 ms and normalized by the expected value of the cross-correlation at 0 time lag under the assumption that the spike trains were independent Poisson processes. This normalization was based on the observed mean firing rates of the two spike trains and their common duration (e.g., if the 2 spike trains were random and uncorrelated, their cross-correlation at 0 time lag would be equal to 1 after normalization). The resulting cross-correlation histograms (cross-correlograms) were smoothed by convolving them with a Gaussian of unit area and a standard deviation of 100 ms. Because periods of rhythmic activity were always much greater than 100 ms, this smoothing preserved all fluctuations in the cross correlograms relevant to the present analysis.

**Cross-correlation of neuronal spike trains**

The data analysis methods we used in previous studies of the rhythmic patterns recorded in single isolated thoracic ganglia relied on our ability to unambiguously identify "bursts" of activity in different sets of motor neurons. The rhythmic patterns were then characterized by comparing the onsets and durations of bursts of activity in different pools of motor neurons (Ryckebusch and Laurent 1993). Because of the irregularity of the activity patterns recorded in chains of two or three thoracic ganglia, these methods were not appropriate for the analysis of the present experiments. It was often difficult to define a burst of activity in a pool of motor neurons because the activity in each pool was irregular, ranging from low-frequency tonic firing of a few motor neurons to high-frequency bursts of action potentials from many motor neurons. A cross-correlation analysis of neuronal spike trains was judged to be more appropriate for these data, because such an analysis could be performed objectively on all of the activity patterns we recorded, irrespective of whether they could be characterized as "rhythmic."

To verify that cross-correlograms were consistent with and comparable with latency histograms, we applied both techniques to a set of extracellular recordings obtained from isolated metathoracic ganglia (Fig. 1). In Fig. 1, three pairs of neuronal spike trains were analyzed with the use of both techniques. Shown in Fig. 1 A is the spike-latency histogram for the right trochanteral depressor *D₄*, relative to the onsets of bursts of activity in different sets of motor neurons. The rhythmic patterns we recorded, irrespective of whether they could be characterized as "rhythmic."

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FIG. 1. Comparison of spike-latency histograms and cross-correlation histograms. Data were obtained from simultaneous recordings from nerves RSA (right trochanteral depressors, CI), L3B (left trochanteral levators), and R3B (right trochanteral levators) of an isolated metathoracic ganglion in 5 × 10⁻⁷ M pilocarpine. In all spike-latency histograms (A, C, and E), spike latencies were computed from the onset of a burst in metathoracic trochanteral levators. In all cross-correlograms (B, D, and F), time lags were relative to action potentials from the metathoracic trochanteral levators used as a reference in the corresponding spike-latency histogram. Cross-correlations were normalized as described in METHODS.

A and B: metathoracic right depressor D₄ (4,764 spikes) relative to metathoracic right levators (53 cycles; 9,049 spikes). C and D: metathoracic right depressor D₄ (4,764 spikes) relative to metathoracic left levators (46 cycles; 6,898 spikes). E and F: metathoracic left levators (46 cycles; 6,898 spikes) relative to metathoracic right levators (53 cycles; 9,049 spikes). Note that, whereas the general shapes of the spike-latency and cross-correlograms are similar, the spike-latency histograms are slightly shifted to the right (discussed in METHODS).

Cross-correlations of two highly structured neuronal spike trains are nonetheless difficult to interpret. Standard statistical tests generally cannot be devised, because the sampling distributions of the spike trains are unknown. Even in the absence of a rigorous statistical test, however, the cross-correlation technique provides powerful information about temporal relationships between neuronal spike trains. As can be seen from the examples shown in Fig. 1, cross-correlograms of spike trains that have been shown by other methods to be interdependent exhibit large peaks and troughs. The following procedure was used to evaluate the significance of modulations in the cross-correlograms.

Only salient features in the cross-correlograms that occurred near 0 time lag (time lag much less than 50% of the estimated cycle period) were examined. The cross-correlogram of two highly structured spike trains typically exhibits many peaks and troughs, particularly if the spike trains are from pacemaker neurons with similar oscillation frequencies (see discussion of Figs. 2 and 3) (and see Perkel et al. 1967). These modulations generally occur at all time lags, and their size relative to significant peaks depends on the amount of noise in the signals and the sample sizes. The cross-correlograms we obtained could be separated into two broad categories. 1) The first category is histograms in which there were no large peaks or troughs near 0 time lag. Although these histograms were often highly structured with many peaks and troughs, the fluctuations were distributed uniformly at all time lags. We never saw a histogram with an isolated peak or trough at a time lag greater than 50% of the average period. These histograms were assumed to show no significant correlations between the spike trains. 2) The second category is histograms with a peak or a trough near 0 time lag that was larger than all other peaks. The correlation or anticorrelation was then confirmed by visual inspection of the extracellular recordings of spike trains from which the data were obtained. Large single peaks at 0 time lag in cross-correlograms always corresponded to visibly synchronized activity of the extracellular spike trains. For example, Fig. 2A shows synchronized activity of the mesothoracic depressor D₄ and prothoracic levators. For every burst of activity of prothoracic levators (middle trace, asterisks), there is a corresponding increase in the spike frequency of mesothoracic D₄ (top trace). The cross-correlogram of these two spike trains is shown in Fig. 2B. Note that this histogram shows a peak at 0 time lag that is larger than any other peaks in the histogram. Figure 2C shows the cross-correlogram of the activity of the prothoracic depressor D₄ (bottom trace) relative to the prothoracic levators. This histogram shows a large trough at 0 time lag, which corresponds to an interruption of the tonic activity of prothoracic D₄ during a prothoracic levator burst (Fig. 2A, middle and bottom traces). The smaller peaks preceding and after the inhibition are less apparent in Fig. 2A, because this is only a small portion of the 250-s recording used to compute the cross-correlogram. (The cross-correlation thus illustrates the general trend in relationships between spike trains and corresponds well to the general impression one receives from visually inspecting large amounts of data, even though this trend may not be apparent in any small subset of data.) Although these criteria were subjective,
The rhythmic activity in leg motor neurons of thoracic ganglia that are completely isolated from each other and from their normal peripheral inputs can be elicited when the preparation is superfused with the muscarinic agonist pilocarpine. These rhythmic patterns have been described in detail elsewhere (Ryckebusch and Laurent 1993; Ryckebusch et al. 1994). In this paper we focus exclusively on the coordination of levators and depressors of the trochanter, whose alternating activity during each cycle describes the walking rhythm well (Ryckebusch and Laurent 1993). The rhythmic activity recorded in each hemiganglion consisted of a short levator phase, during which a brief burst of activity was recorded in trochanteral levator motor neurons, alternating with a longer depressor phase, during which activity was recorded in the slow and fast trochanteral depressors ($D_s$ and $D_f$). During normal walking, these two phases correspond to the swing and stance phases of hind leg stepping, respectively (Burns 1973; Burns and Usherwood 1979). Although alternation of the trochanteral motor neuron antagonists was observed in all three thoracic ganglia, the details of the rhythmic pattern differed (Ryckebusch et al. 1994). In particular, there was an anterior to posterior shift in the activity patterns of the trochanteral depressors relative to the levator bursts. Whereas metathoracic $D_s$ and $D_f$ fired at their highest rates immediately after a trochanteral levator burst, the highest firing frequencies of the prothoracic $D_s$ and $D_f$ occurred before each trochanteral levator burst. The rhythmic activity recorded in the metathoracic ganglion was similar in most respects to that in the
prothoracic ganglion, but $D_s$ and $D_f$ had marked peaks of activity both immediately before and after a levator burst.

**Effects of sectioning thoracic connectives on rhythmic activity**

In preparations consisting of two or three connected thoracic ganglia, the patterns of rhythmic activity were highly variable. Although we could record simultaneous activity of motor neurons in all six hemiganglia, in most preparations rhythmic activity could only be elicited in a subset of these hemiganglia. Rhythmic activity was irregular, and in some hemiganglia only tonic activity was recorded. The average frequencies of rhythms recorded simultaneously in different hemiganglia were generally different. These patterns of activity could change spontaneously during the course of an experiment and could also be modified by altering the bath concentration of pilocarpine.

Sectioning the connectives between ganglia caused large changes in the patterns of activity, such as the emergence of rhythmic activity in previously quiescent hemiganglia. Ongoing rhythmic activity usually became more regular, with more uniform burst shapes and durations and less variable bursting frequencies than were observed before the section. Extracellular recordings and corresponding cross-correlograms for prothoracic levators and mesothoracic depressors before and after sectioning the pro-meso connectives are illustrated in Figs. 2 and 3. The correlated activity between prothoracic levators and mesothoracic depressors is apparent both in the extracellular recordings (Fig. 2A, asterisks) and in the cross-correlogram (Fig. 2B). Sectioning the pro-meso connectives had two major effects: 1) a stable rhythmic pattern emerged in the mesothoracic motor neurons, whereas the prothoracic rhythm was relatively unchanged (Fig. 3A) and 2) evidence of correlated activity in mesotho-
racing depressors and prothoracic levators disappeared (Fig. 3 B). Note that the mesothoracic depressor $D$, no longer fired at a higher frequency during a prothoracic levator burst (Fig. 3 A), as it did before the section (Fig. 2 A); as a result, the large peak at 0 time lag is absent from the post-section cross-correlogram (Fig. 3 B). Figure 3 B also illustrates one of the difficulties of interpreting cross-correlograms of rhythm spike trains. After sectioning the connectives, the pro- and mesothoracic ganglia were rhythmically active with similar average frequencies. Therefore, although the ganglia were isolated from each other and were therefore functionally independent, the cross-correlogram of the activities of prothoracic levators and mesothoracic depressors exhibited regularly spaced peaks. Because these peaks were of uniform amplitude over all time lags, however, they were easily distinguishable from single central peaks.

**Intrasegmental correlations**

Because the rhythmic patterns recorded were irregular, the cross-correlations reported here were not necessarily apparent in all preparations or even at different times during a single experiment. Because the waxing and waning of the correlations between the activities of different motor pools was not predictable and could not be monitored continuously during the course of any experiment, no meaningful quantitative measure of variability could be obtained. The correlations reported here, however, represent those interactions that were observed in most preparations.

**LEVATORS AND CONTRALATERAL DEPRESSORS.** The smallest preparation from which we recorded was a single thoracic ganglion, which is composed of a pair of fused hemiganglia. Coupling was observed between the pattern generators in the two hemiganglia and has been described in detail in our studies of rhythms evoked in isolated metathoracic ganglia (Ryckebusch and Laurent 1993). In particular, we found that the activity of levators of the trochanter was strongly coupled to activity of contralateral depressors of the trochanter (Fig. 1, C and D). There is a large component of trochanteral depressor activity that is synchronized with contralateral trochanteral levator activity. Such coupling between levators and contralateral depressors was also observed in the pro- and mesothoracic ganglia, although not as consistently as in the metathoracic ganglion. Examples of coupling between the activities of levators and contralateral depressors for the pro- and mesothoracic ganglia are shown in Fig. 4, A and B, respectively.

**LEVATORS AND CONTRALATERAL LEVATORS.** Rhythmic activity of trochanteral levators was not strongly coupled to the rhythmic activity of levators in the contralateral hemiganglion; in fact, the intrinsic frequencies of the rhythms on the left and right were generally different. However, levators on opposite sides were never coactive in the metathoracic ganglion (Fig. 1, E and F), and phase-locking between rhythms on the left and right was occasionally observed. In the pro- and mesothoracic ganglia, the coupling between levators on opposite sides was even more variable. Generally, levator activity on opposite sides appeared to be anticorrelated, but in some cases synchronous activity was also observed (Fig. 5). Figure 5 shows cross-correlograms of mesothoracic levators on the left and right sides calculated from four discrete recordings (270–500 s each) in the same experiment. Initially, the levators were anticorrelated, as can be seen from the trough in the cross-correlogram at 0 time lag in Fig. 5 A. Later, the trough at 0 time lag disappeared, and a peak appeared (Fig. 5, B and C). Still later, the left and right levator motor neurons were bursting synchronously (Fig. 5 D). The regularly spaced peaks in the cross-correlograms (e.g., Fig. 5 B) are due to the fact that both motor pools were bursting rhythmically with similar cycle periods (2–3 s). Thus several possible phase relationships occurred between levators on opposite sides of an isolated pro- or mesothoracic ganglion, in contrast to isolated metathoracic ganglia (see above).

**Intersegmental correlations**

**COUPLING BETWEEN LEVATORS IN ADJACENT SEGMENTS.** In most experiments, activity between levators on the same side of different segments appeared to be uncorrelated. Sometimes, however, levator activity in one segment immediately preceded or followed levator activity in an adjacent segment, with a short latency (1–3 s; see below). In the cross-correlograms, this can be seen as a peak near, but not at, 0 time lag (Figs. 6 A and C). In addition, there was often an inhibitory trough in the histogram at 0 time lag (Fig. 7 B).

**Coupling between pro- and mesothoracic levators.** The most commonly seen sequence was activity in prothoracic levators that preceded mesothoracic levator activity (Fig. 6 A), although the reverse was occasionally observed. In some cases, there was also a trough in the cross-correlogram corresponding to anticorrelated activity at 0 time lag (Figs. 6 A and 7 B). This correlation disappeared after the thoracic connectives were sectioned (Fig. 6 B).

**Coupling between meso- and metathoracic levators.** The most commonly seen sequence was activity in mesothoracic levators that followed metathoracic levator activity (Fig. 6 C), although the reverse was occasionally observed. In some cases (not shown), there was strong evidence of anticorrelated activity at 0 time lag (i.e., a trough in the cross-correlogram). Coupling (as evidenced in cross-correlograms) was apparent even when rhythmic activity was not clearly seen in extracellular recordings from one of the hemiganglia. In Fig. 7, for example, bursting activity was recorded in the mesothoracic but not the prothoracic ganglion, but prothoracic activity was clearly modulated during bursts of activity in the mesothoracic levators (Fig. 7 A, asterisks). The trough at 0 time lag and peak in the activity of prothoracic levators preceding mesothoracic levator bursts was similar to cross-correlations from experiments in which both pro- and mesothoracic segments were rhythmically active (Fig. 6 A). After sectioning the pro-meso connectives, the modulation of the activity of prothoracic levators disappeared (Fig. 7 C and D).

**COUPLING BETWEEN LEVATORS AND DEPRESSORS ON THE SAME SIDE OF ADJACENT SEGMENTS.** A burst of activity in trochanteral levators in one segment was correlated with an increase of the firing frequency of trochanteral depressors (usually $D_1$) in the adjacent ipsilateral segment (Fig. 2 A,
top 2 traces). This coupling between levators in one segment and depressors in an adjacent segment was observed in most preparations and appeared to be unaffected by the presence or absence of the third thoracic segment. Cross-correlations of the activity of levators in one segment and depressors in an ipsilateral adjacent segment showed a large peak at 0 time lag. Figure 8 shows cross-correlograms for four different levator/depressor pairs, obtained from different experiments: prothoracic levators and mesothoracic depressors (Fig. 8A); mesothoracic levators and prothoracic depressors (Fig. 8B); mesothoracic levators and metathoracic depressors (Fig. 8C); and metathoracic levators and mesothoracic depressors (Fig. 8D).

Correlations between nonadjacent hemisegments

The significance of correlations between motor neuron activities in nonadjacent hemiganglia was more difficult to assess. This is because, in most cases in which “significant” correlations between two “distant” motor pools were observed, it could be argued that the correlation was an indirect result of shorter-range correlations through an indirect pathway linking the two motor neuron pools. Simultaneous recordings of three motor neuron pools in two hemisegments of the same ganglion (mesothoracic levators and depressors on the left and mesothoracic levators on the right) illustrates this ambiguity (Fig. 9). As previously reported (Ryckebusch et al. 1994), mesothoracic depressors were inhibited during ipsilateral levator bursts and showed increased activity both immediately before and after each levator burst (Fig. 9A). Mesothoracic levators were coactive with contralateral depressors (Fig. 9C; see also Fig. 4B). The cross-correlogram of right and left mesothoracic levators (Fig. 9B) shows inhibition at 0 time lag flanked by excitatory peaks, much like the histogram in Fig. 9A. Taken alone, this histogram would appear to indicate the existence of inhibitory coupling between left and right mesothoracic levators. In the context of the other two histograms (Fig. 9, A and C), however, we could argue that, given that mesothoracic levators and contralateral depressors tended to be coactive (Fig. 9C), the cross-correlogram between left and right levators should resemble the cross-correlogram between left levators and depressors (Fig. 9A). Without additional information, it is not possible to determine which two of the three correlations show significant coupling, or if all three correlations are indicative of independent coupling pathways. In this case we have been able to demonstrate relationships similar to those shown in Fig. 9, A and C, in preparations in which only one of the two mesothoracic hemiganglia was rhythmically active (not shown). This indicates that the coupling between mesothoracic levators and either ipsilateral (Fig. 9A) or contralateral (Fig. 9C) depressors does not depend on the existence of rhythmic contralateral levator activity.

In some preparations, “diagonal” coupling was observed, either between mesothoracic levators and contralateral prothoracic depressors or between prothoracic levators and contralateral metathoracic levators. As discussed above, however, it was not possible in any of these cases to establish whether this coupling was independent of shorter-range interactions. Figure 10 shows pair-wise cross-correlations between right mesothoracic levators and left prothoracic levators (Fig. 10C) along with correlations along a second, indirect pathway (Fig. 10, A and B). Prothoracic levator activity preceded activity in ipsilateral mesothoracic levators (Fig. 10A; see also Fig. 6A), and mesothoracic levators on the right preceded mesothoracic levators on the left (Fig. 10B). These cross-correlations are consistent with
**Fig. 5.** Coupling between left and right mesothoracic levators changes with time. **A:** cross-correlogram of the activity of mesothoracic levators on the left relative to levators on the right. Histogram was computed from 270-s continuous recordings of left (13,890 spikes) and right (8,258 spikes) mesothoracic levators. Pilocarpine $10^{-4}$ M. **B:** as in **A**, later in the experiment, histogram was computed from 297-s continuous recordings of left (13,692 spikes) and right (6,960 spikes) mesothoracic levators. Both sets of motor neurons were rhythmically active with similar periods (2-3 s), giving rise to regular oscillations in the cross-correlogram. **C:** later in the experiment, histogram was computed from 351-s continuous recordings of left (8,204 spikes) and right (5,144 spikes) mesothoracic levators. **D:** later in the experiment, histogram was computed from 506-s continuous recordings of left (10,525 spikes) and right (15,852 spikes) mesothoracic levators. Note that absolute peak amplitudes cannot be compared in **A**–**D**, because the normalization factor for each cross-correlogram was different.

**Fig. 6.** Coupling between adjacent ipsilateral levators. **A:** cross-correlogram of prothoracic levators relative to mesothoracic levators (prothoracic levators precede mesothoracic levators). Histogram was computed from 318-s continuous recordings of mesothoracic (3,552 spikes) and prothoracic (8,797 spikes) levators. Pilocarpine $3 \times 10^{-5}$ M. **B:** same experiment as **A**, after cutting the pro-meso thoracic connectives. Histogram was computed from 192-s continuous recordings of mesothoracic (15,218 spikes) and prothoracic (3,227 spikes) levators. **C:** coupling between meso- and metathoracic levators. Cross-correlogram of mesothoracic levators relative to metathoracic levators (mesothoracic levators follow metathoracic levators). Histogram was computed from 188-s continuous recordings of mesothoracic (2,706 spikes) and metathoracic (634 spikes) levators.

**DISCUSSION**

We have shown that locomotor-like rhythms elicited in leg motor neurons of different hemisegments in isolated thoracic nerve cords are centrally coupled. A summary of the major functional relationships between motor neuron pools as revealed by cross-correlation analysis is shown sche-
Connections between motor neuron pools represent the phase relationships between the motor neurons, not actual neural pathways, which are unknown. Trochanteral levators were coactive with trochanteral depressors in the ipsilateral adjacent hemiganglion and the contralateral hemiganglion of the same segment. Mesothoracic trochanteral levators tended to burst immediately after either pro- or metathoracic levator bursts. The coupling of levators in the two hemiganglia of the same segment was variable over long stretches of time, but not on a cycle-by-cycle basis, particularly in the pro- and mesothoracic ganglia.

**Cross-correlation technique**

**Statistical significance.** We have used cross-correlation analysis to assess functional relationships between motor neuron pools in different segments of isolated thoracic nerve cords. Although this technique was particularly well-suited to exhibit the interdependencies between highly irregular spike trains, the interpretation of our results was restricted by the inherent limitations of the cross-correlation method. The question of how to determine whether a structure in a cross-correlogram is statistically significant has often been raised, but so far no generally recognized solution has been presented (Aertsen et al. 1989; Glaser and Ruchkin 1976; Perkel et al. 1967). One is left with the empirical procedure of comparing a peak or a trough near 0 time lag with the magnitudes of fluctuations in eccentric positions of the correlograms. This is potentially problematic when cross-correlating the activities of neurons with pacemaker-like properties, because the resulting histograms often show large and regular modulations, even when those neurons are known to be independent (Perkel et al. 1967). These limitations can be overcome, however, if cross-correlation is combined with other analysis techniques and is interpreted cautiously (see METHODS).

**Neuronal connectivity.** One goal of cross-correlation analysis is to derive information on potential neuronal connectivity from physiological data. Two neurons receiving common excitatory inputs from unknown sources will have more synchronous spikes than statistically expected if they did not, and their cross-correlation function will correspondingly show a central peak. Although it is less obvious, common inhibitory inputs will also tend to produce a central peak in the cross-correlogram. Likewise, several circuit configurations can result in a central trough in the cross-correlogram. Accordingly, although a central peak or trough in the cross-correlogram is indicative of common inputs, it is impossible to determine uniquely the underly-
Intersegmental coordination and the implications for behavior

We have shown that, in isolated thoracic nerve cords, central mechanisms exist that coordinate the leg motor patterns of the different thoracic segments. Furthermore, similarities between the intersegmental coupling reported here and results obtained in intact and deafferented insects indicate that this central coupling between leg motor neuron pools may be one of the mechanisms involved in the coordination of the legs during walking. In several arthropod preparations, segments on the same side of the body seem to be more strongly coupled than those on opposite sides (Dean 1989; Pearson and Iles 1973; Sillar et al. 1987). Similarly, although we found evidence for both ipsilateral and contralateral intersegmental coupling, the strongest interactions appeared to be between segments on the same side. The pair-wise interactions we analyzed were of two types: interactions between motor neurons of the same motor pool in different hemisegments (levator-levator) and interactions between antagonistic motor neuron pools in different hemisegments (levator-depressor). The levators-depressor interactions were stronger and less variable than were levator-levator interactions.

LEVATOR-LEVATOR COUPLING. That levator motor neurons on opposite sides were never coactive in the metathoracic ganglion suggests that there could be inhibitory coupling between the circuits that drive left and right levators. In contrast, levator motor pools in the pro- and mesothoracic ganglia could be either in antiphase or in phase and could be successively in and out of phase during the course of one experiment (Fig. 5). Such a variable pattern of coordination suggests that the coupling between levator pools may be modulated during walking to modify the coordination pattern of the legs. In other systems, particularly the crustacean stomatogastric nervous system, which controls movements of the foregut, it has been shown that the phase relationships between elements of a central pattern generator are subject to modification by endogenous neuromodulators (Marder and Weimann 1992). The variable nature of the coordination indicates that sensory inputs must play an important role in determining the sequence of leg protractions. The in-phase pattern of coordination sometimes observed is reminiscent of observations in walking stick insect, in which simultaneous protractions of the left and right legs could be maintained for several cycles (Graham 1972).

The coupling observed between levators in adjacent he-
mismatches on the same side was similar to that seen in deafferented headless cockroaches (Pearson and Iles 1973), in which mesothoracic bursts tended to occur immediately after the end of the metathoracic burst. Pearson and Iles (1973) suggested that a nonoverlapping sequence of levator bursts could be generated by inhibitory coupling followed by a postinhibitory rebound mechanism. In fact, inhibitory coupling followed by asymmetric delayed excitation between ipsilateral adjacent legs has been demonstrated in the stick insect, but it is not known whether this coupling is achieved by sensory or central mechanisms, or a combination of the two (Cruse 1990).

**LEVATOR-DEPRESSOR COUPLING.** Previous results (Ryckebusch and Laurent 1993; Pearson and Iles 1970) indicate that the drives to levator and depressor motor neurons are not symmetrical. The occurrence of levator bursts appeared to be independent of the tonic firing rate of depressors or the presence or absence of depressor bursts timed with the rhythmic activity of other segments. Depressor activity, on the other hand, appeared to be modulated in time with the rhythmic activity of contralateral or ipsilateral levators, because an increase in depressor activity was always coupled to a contralateral levator burst or appeared in conjunction with an ipsilateral levator burst, and depressor activity was never inhibited in the absence of a corresponding ipsilateral levator burst. In isolated ganglia in which there was no rhythmic activity in levators on either side, only tonic activity was recorded in depressors.

Mechanisms proposed by Pearson and Iles (1970) based on their studies of leg motor rhythms in the cockroach are not sufficient to account for the asymmetric coupling between trochanteral levators and depressors we observed. They propose that a central pattern generator simultaneously excites the motor neurons active during the protraction phase (here, trochanteral levators) and inhibits antagonistic motor neurons active during the retraction phase (trochanteral depressors). A mechanism such as postinhibitory rebound was postulated to explain the postinhibitory burst of activity in depressors. This mechanism, however, cannot account for the observation in pro- and mesothoracic ganglia that depressors were also active before being inhibited during a levator burst. In a modeling study, we proposed that this result could be explained by concurrent excitation of both levators and depressors (Ryckebusch et al. 1994). In addition, because depressors appear to receive excitatory inputs during levator bursts in adjacent hemisegments, an additional excitatory drive to depressors from central pattern generators in adjacent segments would also

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**FIG. 9.** Coupling between mesothoracic trochanteral levators and depressors. Cross-correlograms from 270-s simultaneous recordings of mesothoracic levators (13,890 spikes) and depressors (3,201 spikes) on the left, and levators on the right (8,258 spikes). Pilocarpine $10^{-3}$ M. A: cross-correlogram of levators on the left relative to depressors on the left. B: cross-correlogram of levators on the right relative to levators on the left. C: cross-correlogram of levators on the right relative to depressors on the left.
be required. Evidence that intersegmental coordinating inputs are physically active in the levator phase was obtained by Pearson and Iles (1973) in cockroaches. Recording from axons in the connectives that were rhythmically active in phase with walking rhythms, they reported that such axons always discharged in phase with levators in either segment and never with depressors.

The strong centrally mediated coordination between contralateral or adjacent levators and depressors stands in contrast to the weaker, variable coupling between levators. Because the legs of a locust must be capable of relatively independent movement to navigate on irregular terrain, it is sensible that leg protractions (levator phase) should be only weakly coupled centrally, relying more heavily on sensory information to achieve proper coordination. Leg protraction is potentially destabilizing, however, because weight is then shifted to other legs. Regardless of the sequence of leg protractions, stability must be maintained each time a leg is lifted by stiffening the joints of the remaining legs to bear the increased load and possibly preventing the protraction of adjacent legs. In the cockroach, increased load on a leg was shown to cause an increase in the firing rate of the slow depressor \( D_s \) (Pearson 1972). It is therefore likely that the increased activity of depressors (particularly the slow depressor \( D_s \)) during levator bursts in adjacent hemisegments at least partially subserves such a function. It has been assumed that load compensation in insects was mediated by feedback from sensory organs (Pearson 1972; Zill and Moran 1981). On the basis of our results, it appears likely that a central mechanism for maintaining stability exists as well.

**Neural basis of intersegmental coordination**

**Coupling between segmental pattern generators.** Motor neurons are driven either directly by central pattern generator neurons, or by other neurons that are themselves phasically driven by a central pattern generator. Because direct connections between leg motor neurons have been reported only exceptionally in insects (Burrows et al. 1989; Siegler 1982), central coupling between pools of motor neurons must be mediated by premotor interneurons. Two coupling mechanisms can be distinguished. 1) Coordinating inputs carrying phasic information from one segmental pattern generator could be directly involved in shaping the rhythmic output of a pattern generator in another segment.
INTERSEGMENTAL COORDINATION OF LOCUST LEG CPGs

In this case the rhythms generated by two connected segmental pattern generators would not be independent. 2) The motor neurons (or interneurons that drive them) are simultaneously receiving independent phasic inputs from several uncoupled segmental pattern generators. In this case the output pattern of a motor neuron would represent the superposition of its different phasic inputs, which are themselves uncoordinated in the absence of sensory feedback. The fact that severing thoracic connectives leads to large changes in segmental rhythms, in most cases causing the rhythmic patterns to become more regular, indicates that the premotor circuits driving motor neurons are likely to be coupled. Weak central coupling between segmental pattern generators and the absence of additional coordinating inputs from sensory pathways could explain the highly irregular rhythmic patterns we observed. In the absence of sensory feedback, rhythmic inputs from one pattern generator to the next would occur at inappropriate times during the cycle, causing a disruption of otherwise “regular” rhythmic patterns. Although motor neurons may be getting additional phasic inputs from other segments, it seems likely that the rhythmic output of a central pattern generator in one hemisegment is shaped in part by phasic inputs from other segments.

INTERSEGMENTAL INTERNEURONS. Intersegmental interneurons have been implicated in the coordination of motor behaviors in a number of insect preparations. In cockroaches a large population of intersegmental interneurons (type-A thoracic interneurons or TLs) has been identified that integrate inputs from different sources and project to local premotor and motor neurons in several ganglia, coordinating leg movements during the escape reflex (Ritzmann and Pollack 1986, 1988, 1990; Ritzmann et al. 1991). In addition, Pearson and Iles (1973) recorded from axons in the thoracic connectives whose activity patterns were modulated in phase with centrally generated rhythms in the ipsilateral adjacent segments. The pattern generator underlying locust flight, which can produce well-coordinated motor patterns in the absence of sensory inputs, is distributed in all three thoracic ganglia and includes many intersegmental interneurons (Robertson and Pearson 1983; review: Robertson 1986; but see Ronacher et al. 1988). In stick insects it has been established that the various mechanisms responsible for the coordination of adjacent legs (Cruse 1990) are mediated primarily by neural signals traveling in the ipsilateral connectives (Dean 1989). It is not known, however, whether these coordinating signals are driven by sensory or central inputs. The neuronal basis of central coordination between pattern generators underlying leg movements in the locust is unknown, although several populations of intersegmental interneurons have been studied. In one study, at least 30 paired intersegmental interneurons with somata in the mesothoracic ganglion were shown to receive mechanosensory inputs from the middle leg and to project to the ipsilateral metathoracic hemiganglion, where they made synaptic contacts with the local interneurons and motor neurons controlling the hind leg (Laurent 1986-1988; Laurent and Burrows 1989a,b). In another study, ~35 interneurons with somata in the metathoracic ganglion were shown to receive mechanosensory inputs from the hind leg and project to the mesothoracic ganglion in either the ipsilateral or the contralateral connective (Laurent and Burrows 1988). Although it is not known whether these neurons receive central inputs from local circuits controlling the legs in addition to the sensory inputs, their anatomic and physiological characteristics make them good candidates for the role of intersegmental coordinating interneurons.

Concluding remarks

The neuronal components of the pattern generators underlying leg movements have not been identified, and studies of intersegmental interneurons in insects suggest that the number of neurons involved in the coordination of these local circuits is likely to be very large (Laurent 1987; Laurent and Burrows 1988; Ritzmann and Pollack 1988; Rowell 1993). The results presented here can guide further exploration of the nature of the central coupling between segmental pattern generators, both through additional experiments and through the use of computer models to test possible circuit designs. In a parallel study, we proposed a model for a hemisegmental pattern generator that could be
configured to generate different motor patterns appropriate for each pair of legs (Ryckebusch et al. 1994). These hemisegmental models could now be connected into a larger circuit to test our hypotheses about the central coupling between pattern generators.

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